PREPARED FOR

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

MANNED SPACECRAFT CENTER

LUNAR RECEIVING LABORATORY

0051

TEST PLAN

CREW MICROBIOLOGY EVALUATION FOR APOLLO MISSION 101

LRL/BRN TEST NUMBER: 68-138

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I. LRL/BRN TEST NO: 68-138

II. TITLE: Crew Microbiology Evaluation for Apollo Mission 101

III. OBJECTIVES:

A. To evaluate microbiologically at 30 and 14 days preflight, immediate preflight and postflight, and 7 days postflight, samples from the crew of Apollo Mission 101.

IV. PROJECT ORIGINATOR: J. K. Ferguson, Ph.D.

TEST OFFICER: C. P. Truby, Ph.D.

V. REFERENCES:

J. K. Ferguson, "Microbiological Assessment of the Crew, Hardware and Clothing for the CRA"; C. P. Truby, "Assessment of Crew Microbiology Protocol," LRL/BRN No. 67-6 and "Crew Microbiology Evaluation for [illegible]" LRL/BRN Test No. 68-29.

VI. BACKGROUND:

This study was initiated in order to evaluate the microbiological profiles of crew members from Apollo Earth-Orbital Mission 101. This effort is necessary in order to (1) determine if pathogenic organisms are present at preflight and postflight sampling times, (2) determine the effects of space flight on the microbiological flora of an astronaut, (3) catalog data of the normal flora of astronauts so that possible human contaminates can be isolated and identified during the Apollo mission to the moon.

VII. METHODS OF TEST:

Division I Bacteriology

Division II Mycology

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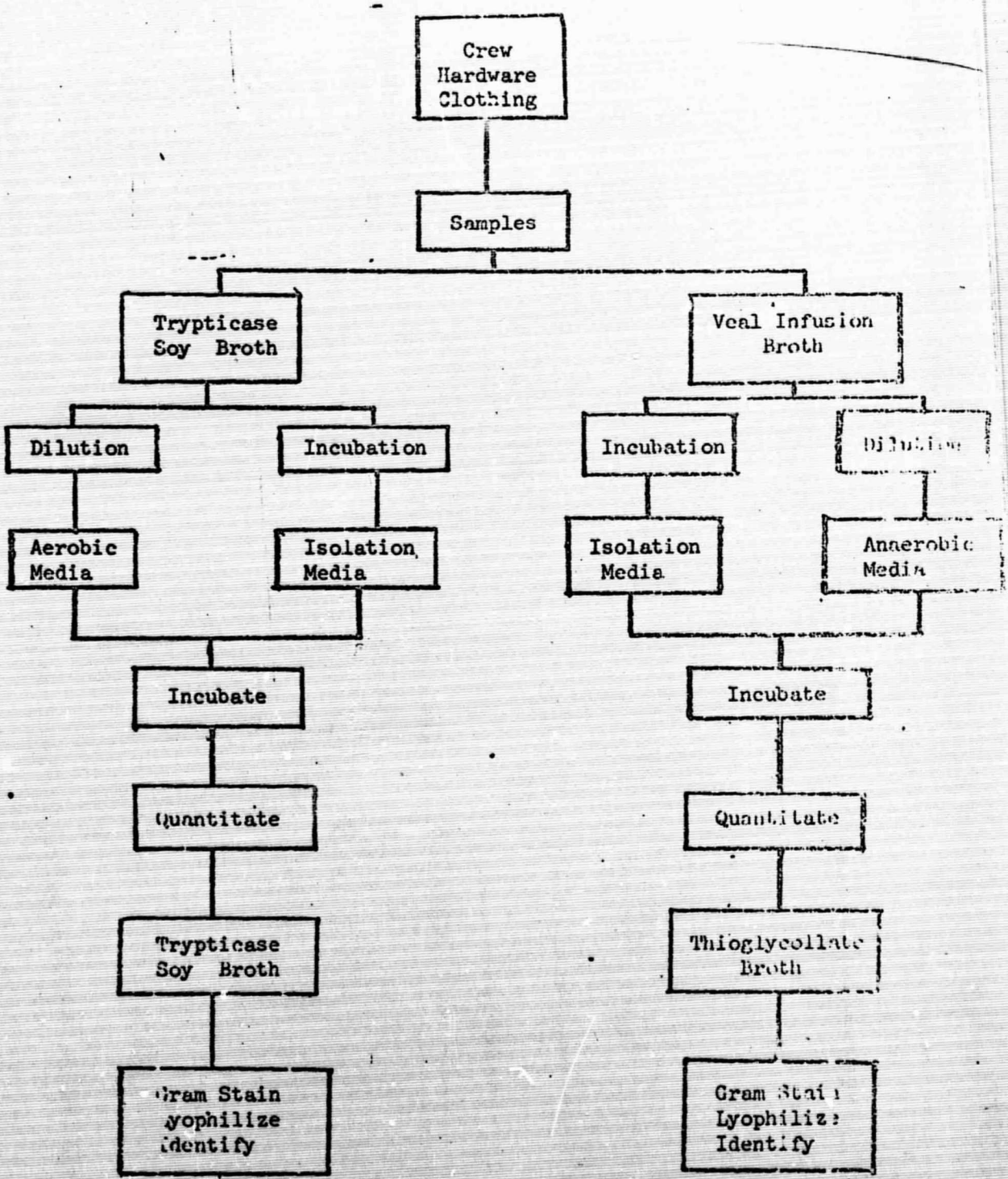
OUTLINE OF BACTERIOLOGY ANALYSES



Division I

Bacteriology

A. Sample Areas: All microbiological samples will be obtained from the three crew members of Apollo Mission 101, the Apollo Spacecraft Command Module, and the clothing of the three astronauts.

1. Crew Microbiology: Eleven samples will be taken from each crew member at the designated times.

a. External swabs: Two calcium alginate swabs (dampened with phosphate buffer) will be taken from each designated area. One swab will be placed in a screw-cap tube containing 10.0 ml of sterile Trypticase Soy Broth (TSB). The second swab will be placed in a screw-cap tube containing 10.0 ml of sterile Veal Infusion Broth (VIB). The broth tubes will be maintained at 4 C during transportation to the laboratory and dilution procedures.

(1) Scalp: An area two square inches two inches up from the hairline at the base of neck will be sampled with two swabs.

(2) External auditory canals: The right and left auditory canals will be sampled with each of two swabs. At least two revolutions will be made with each swab in each canal.

(3) Axillae: An area one square inch below hair area of the left and right axillae will be sampled with each of two swabs.

(4) Umbilicus: The internal area of the umbilicus, and a

surrounding two square inch area will be sampled with two swabs. At least two revolutions will be made with each swab.

(5) Inguinal region: A two inch strip from front to rear on the left and right groin areas between the legs will be sampled with each of two swabs.

(6) Toe webs: An area between the large and first toe of the right and left foot will be sampled with each of two swabs.

(7) Hands: An area of one square inch on the right and on the left hand palms will be sampled with each of two swabs.

b. Nasal passages: Both nostrils of each crew member will be sampled with each of two swabs. One swab will be placed in a screw-cap tube containing 10.0 ml of sterile TSB. The second swab will be placed in a screw-cap tube containing 10.0 ml of sterile VIB. The broth tubes will be maintained at 4 C during transportation to the laboratory and dilution procedures.

c. Throat-Mouth Gargle:

(1) Each crew member will gargle with 60.0 ml of Phosphate Buffer.

(2) The gargle wash will be rinsed through the oral cavity three times.

(3) The wash will be emptied into a wide-mouth bottle containing 20 ml of quadruple strength Tryptose Phosphate Buffer.

(4) The wash containers will be maintained at 4 C during transportation to the laboratory and dilution procedures.

d. Urine: A mid-stream urine sample will be taken from each crew member. Sixty milliliters of urine will be collected in a sterile container. The urine will be maintained at 4 C during transportation to the laboratory and dilution procedures.

e. Feces: A stool sample from each crew member will be obtained in a stool Collection Device as near to each designated sampling time as possible. The stool samples will be stored under an atmosphere of hydrogen and at 4 C during transportation to the laboratory.

2. Spacecraft Hardware Microbiology: Four samples will be taken from the Command Module Hardware at the designated times. Two calcium alginate (dampened with phosphate buffer) swabs will be used to sample each designated area. One swab will be placed in a screw-cap tube containing 5.0 ml of sterile TSB. The second swab will be placed in a screw-cap tube containing 5.0 ml of sterile VIB. The broth tubes will be maintained at 4 C during transportation to the laboratory and dilution procedures.

a. Floor: An area two square inches on the floor of the spacecraft will be sampled with two swabs.

b. Maneuvering Knob: An area two inches along the top half of the maneuvering knob will be sampled with two swabs.

c. Drink-gun: An area completely around the drink-gun orifice will be sampled with two swabs.

3. Astronaut Clothing Microbiology: Two samples will be taken from each crew member suit at the designated times. Two calcium alginate swabs (dampened with phosphate buffer) will be used to sample each designated area. One swab will be placed in a screw-cap tube containing 5.0 ml of sterile TSB. The second swab will be placed in a screw-cap tube containing 5.0 ml of sterile VIB. The broth tubes will be maintained at 4 C during transportation to the laborstory and dilution procedures.

a. Gloves: An area of one square inch on the right and on the left hand gloves will be sampled with each of two swabs.

b. Shoe Soles: An area of one square inch on the right and on on the left shoe soles will be sampled with each of two swabs.

B. Sampling Times:

1. Crew Microbiology: The astronauts will be sampled at the following times: 30 days preflight

14 days preflight

Immediate preflight

Immediate postflight

7 days postflight

Samples from the 30 day, immediate preflight and immediate postflight will be analyzed according to the procedures outlined in the text of this test plan. The 14 day preflight and 7 day postflight samples will be analyzed as outlined in Appendix I.

2. Spacecraft Hardware Microbiology: The Command Module Hardware will be sampled immediate preflight and immediate postflight. Samples will be analyzed according to the procedures outlined in

the text of this test plan.

3. Astronaut Clothing Microbiology: The astronaut clothing will be sampled immediate preflight and immediate postflight. Samples will be analyzed according to the procedures outlined in the text of this test plan.

C. Dilution, Plating and Quantitative Determination:

1. External swabs: The scalp, external auditory canal, axilla, umbilicus, inquinal region, toe web, and hand samples will be treated as follows:

a. Dilution: All TSB sample tubes used for aerobic identification and quantitation will be serially diluted in sterile TSB. All VIB sample tubes used for anaerobic identification and quantitation will be serially diluted in sterile VIB. The sample and dilution tubes will be maintained at 4 C employing an ice bath during the dilution procedures.

(1) The sample TSB and VIB tubes will be vortexed for 5 seconds.

(2) Serial dilutions will be prepared by transferring 1.0 ml aliquots to 9.0 ml of sterile TSB or VIB.

(3) The samples will be diluted in TSB and VIB as follows: the 10^1 dilution represents the first dilution after the sample tube.

(a) Scalp: TSB $.0^1$ to 10^4

VIB 10^1 to 10^4

(b)	External auditory canal:	TSB 10^1 to 10^4
		VIB 10^1 to 10^4
(c)	Axilla: -	TSB 10^1 to 10^4
		VIB 10^1 to 10^4
(d)	Umbilicus:	TSB 10^1 to 10^4
		VIB 10^1 to 10^4
(e)	Inquinal region:	TSB 10^1 to 10^5
		VIB 10^1 to 10^5
(f)	Toe web:	TSB 10^1 to 10^4
		VIB 10^1 to 10^4
(g)	Hand:	TSB 10^1 to 10^3
		VIB 10^1 to 10^3

b. Plating: One-tenth milliliter will be aseptically transferred from each sample and dilution TSB tube to the aerobic quantitative agar media. One-tenth milliliter will aseptically transferred from each sample and dilution VIB tube to the anaerobic quantitative agar media. The agar plates will be spread with a rod.

(1) The aerobic quantitative media for the External swabs include:

(a) Blood agar (BA)

(b) Staphylococcus-110 agar (S-110)

(2) The anaerobic quantitative media for the External swabs include:

(a) Blood agar with vitamin K and Hemin

(3) Four milliliters from each TSB sample tube will be aseptically transferred to a labelled sterile screw-cap for mycobiological analysis.

c. Incubation and Quantitation:

(1) The aerobic quantitative media will be incubated at 35 C for 48 hours.

(2) The anaerobic quantitative media will be incubated at 35 C for 96 hours under an atmosphere of hydrogen gas.

(3) Colony counts will be performed on all quantitative media after incubation.

d. Isolation Streaks:

(1) After mycological samples have been removed from the TSB sample tubes, the TSB & VIB sample tubes will be incubated for 24 hours at 35 C. In addition, all TSB and VIB dilution tubes will be incubated for 24 hours at 35 C and stored at 4 C for 7 days.

(2) After incubation a loop will be used to transfer culture from each sample tube to the isolation media. An isolation streak will be made on each medium.

(3) The isolation media used for the Crew External samples include:

(a) Blood agar

(b) Staphylococcus-110 agar

(c) MacConkey agar (MAC)

(d) Blood agar with vitamin K and Hemin (Anaerobic)

(4) The streaked isolation media will be incubated for 48 or 96 hours at 35 C under the appropriate atmosphere.

(5) After the crew sample tubes have been employed for quantitation and isolation they will be prepared for lyophilization procedures.

2. Nasal Passages: Samples from the nasal passages will be treated as follows:

a. Dilution: All TSB sample tubes used for aerobic identification and quantitation will be serially diluted in sterile TSB. All VIB sample tubes used for anaerobic identification and quantitation will be serially diluted in sterile VIB. The sample and dilution tubes will be maintained at 4 C employing an ice bath during the dilution procedures.

(1) The sample TSB and VIB tubes will be vortexed for 5 seconds.

(2) Serial dilutions will be prepared by transferring 1.0 ml aliquotes to 9.0 ml of sterile TSB or VIB.

(3) The nasal samples will be diluted in TSB and VIB as follows: the 10^1 dilution represents the first dilution after the sample tube.

TSB $10^1 - 10^4$

VIB $10^1 - 10^4$

b. Plating: One-tenth milliliter will be aseptically transferred from each sample and dilution TSB tube to the aerobic quantitative agar media. One-tenth milliliter will be aseptically transferred from each sample and dilution VIB tube to the

anaerobic quantitative agar media. The agar plates will be spread with a glass rod.

(1) The aerobic quantitative media for the nasal passage swabs include:

(a) Blood agar

(b) Staphylococcus-110 agar

(2) The anaerobic quantitative media for the nasal passage swabs include:

(a) Blood agar with vitamin K and Hemin

(b) Paromomycin - Vancomycin - Menadione agar (PVM)

(c) Rogosa agar (Rogosa)

(3) Four milliliters from each TSB sample tube will be aseptically transferred to a labelled sterile screw-cap tube tube for mycobiological analysis.

c. Incubation and Quantitation:

(1) The aerobic quantitative media will be incubated at 35 C for 48 hours.

(2) The anaerobic quantitative media will be incubated at 35 C for 96 hours under an atmosphere of hydrogen gas.

(3) Colony counts will be performed on all quantitative media after incubation.

d. Isolation Streaks:

(1) After mycological samples have been removed from the TSB sample tubes, the sample TSB and VIB tubes will be incubated for 24 hours at 35 C. In addition, all TSB and VIB dilution tubes will be incubated for 24 hours at 35 C

and stored at 4 C for 7 days.

(2) After incubation a loop will be used to transfer culture from each sample tube to the isolation media. An isolation streak will be made on each medium.

(3) The isolation media used for the nasal passage samples include:

(a) Blood agar

(b) Staphylococcus-110 agar

(c) MacConkey agar

(d) Chocolate agar (CHOC)(CO_2)

(e) Blood agar with vitamin K and Hemin (Anaerobic)

(f) Paromomycin - Vancomycin - Menadione agar (Anaerobic)

(g) Rogosa agar (Anaerobic)

(4) The streaked isolation media will be incubated for 48 or 96 hours at 35 C and under the appropriate atmosphere. The media incubated under CO_2 (Chocolate agar) will be placed in an incubator with a CO_2 concentration of 8-10%.

(5) After the nasal sample tubes have been employed for quantitation and isolation they will be prepared for lyophilization procedures.

3. Throat - Mouth Gargle: Samples from the throat-mouth gargle will be treated as follows:

a. Dilution: All throat-mouth gargle samples will be diluted in sterile TSB or VIB for aerobic and anaerobic quantitation. The sample and dilution tubes will be maintained at 4 C em-

ploying an ice bath during the dilution procedures.

(1) The throat-mouth gargle sample will be swirled gently.

(2) Serial dilutions will be prepared by transferring 1.0 ml aliquotes to 9.0 ml of sterile TSB or VIB.

(3) The throat-mouth gargle samples will be diluted in TSB or VIB as follows: the 10^1 dilution represents the first dilution after the sample bottle. TSB 10^1 - 10^5

VIB 10^1 - 10^5

b. Plating: One-tenth milliliter will be aseptically transferred from each sample bottle and dilution TSB tube to the aerobic quantitative agar media. One-tenth milliliter will be aseptically transferred from each sample bottle and dilution VIB tube to the anaerobic quantitative agar media. The agar plates will be spread with a glass rod.

(1) The aerobic quantitative media for the throat-mouth gargle sample include:

(a) Blood agar

(b) Staphylococcus-110 agar

(c) Mitis Salivarius agar (MSA)

(2) The anaerobic quantitative media for the throat-mouth gargle sample include:

(a) Blood agar with Vitamin K and Hemin

(b) Paromomycin - Vancomycin - Menadione agar

(c) Rogosa agar

(3) Four milliliters from each sample bottle and all TSB dilution tubes will be aseptically transferred to indi-

vidually labelled sterile screw-cap tubes for mycobiological analysis.

c. Incubation and Quantitation:

(1) The aerobic quantitative media will be incubated at 35 C for 48 hours.

(2) The anaerobic quantitative media will be incubated at 35 C for 96 hours under an atmosphere of hydrogen gas.

(3) Colony counts will be performed on all quantitative media after incubation.

d. Isolation Streaks:

(1) After mycological samples have been removed from the sample bottles, the sample bottles will be incubated for 24 hours at 35 C. In addition, all TSB and VIB dilution tubes will be incubated for 24 hours at 35 C and stored at 4 C for 7 days.

(2) After incubation a loop will be used to transfer culture from each sample bottle to the isolation media. An isolation streak will be made on each medium.

(3) The isolation media used for the throat-mouth gargle samples include:

(a) Blood agar

(b) Staphylococcus-110agar

(c) MacConkey agar

(d) Chocolate agar (CO_2)

(e) Fildes Enrichment agar (FEA)

(f) Blood agar with vitamin K and Hemin (Anaerobic)

(g) Paromomycin - Vancomycin - Menadione agar (Anaerobic)

(h) Rogosa agar (Anaerobic)

(4) The streaked isolation media will be incubated for 48 or 96 hours at 35 C under the appropriate atmosphere. The media incubated under CO_2 (Chocolate agar) will be placed in an incubator with a CO_2 concentration of 8-10%.

(5) After the throat-mouth gargle samples have been employed for quantitation and isolation they will be prepared for lyophilization procedures.

4. Urine: Samples of the urine will be treated as follows:

a. Dilution: All urine samples used for aerobic identification and quantitation will be serially diluted in sterile TSB. All VIB urine samples used for anaerobic identification and quantitation will be serially diluted in sterile VIB. The urine samples and dilution tubes will be maintained at 4 C employing an ice bath during the dilution procedures.

(1) The urine sample containers will be swirled gently.

(2) Serial dilutions will be prepared by transferring 1.0 ml aliquots to 9.0 ml of sterile TSB or VIB.

(3) The urine samples will be diluted in TSB and VIB as follows: the 10^1 dilution represents the first dilution after the sample tube: TSB 10^1 to 10_3

VIB 10^1 to 10_2

b. Plating: One-tenth milliliter will be aseptically transferred from each urine sample and dilution TSB tube to the aerobic quantitative agar media. One-tenth milliliter will be aseptically transferred from each urine sample and dilution VIB tube to the anaerobic quantitative agar media. The agar plates will be spread with a glass rod.

(1) The aerobic quantitative media for the urine samples include:

(a) Blood agar

(b) Staphylococcus-110 agar

(c) MacConkey agar

(2) The anaerobic quantitative media for the urine samples include:

(a) Blood agar with vitamin K and Hemin

(b) Rogosa agar

(3) Four milliliters from each sample bottle will be aseptically transferred to a sterile screw-cap tube for mycological analysis.

c. Incubation and Quantitation:

(1) The aerobic quantitative media will be incubated at 35 C for 48 hours.

(2) The anaerobic quantitative media will be incubated at 35 C for 96 hours under an atmosphere of hydrogen gas

(3) Colony counts will be performed on all quantitative media after incubation.

d. Isolation Streaks:

(1) After mycological samples have been removed from the urine samples, the urine samples will be incubated for 24 hours at 35 C. In addition, all TSB and VIB dilution tubes will be incubated for 24 hours at 35 C and stored at 4 C for 7 days.

(2) After-incubation a loop will be used to transfer cultures from each urine sample to the isolation media. An isolation streak will be made on each medium.

(3) The isolation media used for the urine samples include:

(a) Blood agar

(b) Staphylococcus-110 agar

(c) MacConkey agar

(d) Blood agar with vitamin K and Hemin (Anaerobic)

(e) Rogosa agar (Anaerobic)

(4) The streaked isolation media will be incubated for 48 or 96 hours at 35 C under the appropriate atmosphere.

(5) After the urine samples have been employed for quantitation and isolation they will be prepared for lyophilization procedures.

5. Feces: Samples of feces will be treated as follows:

a. Dilution: All stool sample used for aerobic identification and quantitation will be serially diluted in sterile TSB. All stool sample used for anaerobic identification and quantitation will be serially diluted in sterile VIB. The dilution

tubes will be maintained at 4 C employing an ice bath during the dilution procedures. A Formalin - Ether preparation of each stool sample for ova, cysts, and parasites will be performed.

(1) One-tenth gram from the center of the stool sample will be weighed onto inert weighing paper and transferred to 9.9 ml of sterile TSB.

(2) One-tenth gram from the center of the stool sample will be weighed onto inert weighing paper and transferred to 9.9 ml of sterile VIB.

(3) The TSB and VIB tubes containing the weighed stool samples will be vortexed for 30 seconds.

(4) Serial dilutions will be prepared by transferring 1.0 ml aliquotes to 9.0 ml of sterile TSB or VIB.

(5) The samples will be diluted in TSB or VIB as follows:

TSB $10^{1} - 10^{6}$

VIB $10^{1} - 10^{8}$

b. Plating: One-tenth milliliter will be aseptically transferred from each dilution TSB tube to the aerobic quantitative agar media and Tetrathionate Broth (TB). One-tenth milliliter will be aseptically transferred from the $10^{3} - 10^{8}$ dilution VIB tubes to the anaerobic quantitative agar media. The agar will be spread with a glass rod.

(1) The aerobic quantitative media for the stool samples include:

(a) Blood agar

(b) MacConkey agar

(c) Mitis - Salivarius agar

(d) Tetrathionate Broth

(2) The anaerobic quantitative media for the vaginal samples include:

(a) Blood agar with vitamin K and Hemin

(b) Paromomycin - Vancomycin - Menadione agar

(c) Rogosa agar

(3) Four milliliters from each TSB enrichment will be aseptically transferred to a labelled sterile screw cap tube for mycobiological analysis.

c. Incubation and Quantitation:

(1) The aerobic quantitative media will be incubated at 35 C for 48 hours.

(2) The anaerobic quantitive media will be incubated at 35 C for 96 hours under an atmosphere of [illegible]

(3) The Tetrathionate Broth will be incubated [illegible] hours at 35 C.

(4) Colony counts will be performed on all appropriate plates after incubation.

d. Isolation Streaks:

(1) After mycological samples have been [illegible] 10^1 TSB dilution tubes, the 10^1 TSB and 10^1 VIF dilution tubes will be incubated for [illegible]

addition, all TSB and VIB dilution tubes will be incubated for 24 hours at 35 C and stored at [illegible] for 7 days.

(2) After incubation a loop will be used to transfer the culture from each sample tube to the isolation media. One loopfull of Tetrathionate culture will be used to inoculate the Salmonella - Shigella agar. An isolation streak will be made on each medium.

(3) The isolation media used for the stool samples include:

(a) Blood agar

(b) MacConkey agar

(c) Mitis - Salivarius agar

(d) Salmonella - Shigella agar (SS)

(e) Blood agar with vitamin K and Hemin (Anaerobic)

(f) Paromomycin - Vancomycin - Menadion agar (Anaerobic)

(g) Egg Yolk agar (EYA) (Anaerobic)

(h) Rogosa agar (Anaerobic)

(4) The streaked isolation media will be incubated for 48 or 96 hours at 35 C under the appropriate atmosphere.

(5) After the 10^1 TSB and VIB dilution tubes have been employed for quantitation and isolation they will be prepared for lyophilization procedures.

6. Hardware and Clothing: The Spacecraft Floor, Mattress Pad, Knob, Drink-gun, Urine Collection Device, and the Astronaut Clothing Gloves and Shoe Soles will be treated as follows:

a. Dilution: All TSB sample tubes used for aerobic [illegible]

cation and quantitation will be serially diluted in sterile VIB. The sample and dilution tubes will be maintained at 4 C employing an ice bath during the dilution procedures.

(1) The sample TSB and VIB tubes will be vortexed for 5 seconds.

(2) Serial dilutions will be prepared by transferring 1.0 ml aliquotes to 9.0 ml of sterile TSB or VIB.

(3) The preflight samples will be diluted in TSB and VIB as follows: the 10^1 dilution represents the first dilution after the sample tube:

(a)	Floor:	TSB 10^1 - 10^2
		VIB 10^1 - 10^2
(b)	Maneuvering Knob:	TSB 10^1 - 10^2
		VIB 10^1 - 10^2
(c)	Drink-Gun:	TSB 10^1 - 10^2
		VIB 10^1 - 10^2
(d)	Urine Collection Device:	TSB 10^1 - 10^2
		VIB 10^1 - 10^2
(e)	Gloves:	TSB 10^1 - 10^2
		VIB 10^1 - 10^2
(f)	Shoe Soles:	TSB 10^1 - 10^2
		VIB 10^1 - 10^2

(4) The postflight samples will be diluted an additional 2 logs in TSB and VIB.

b. Plating: One-tenth milliliter will be aseptically transferred

from each sample and dilution TSB tube to the aerobic quantitative agar media. One-tenth milliliter will be aseptically transferred from each sample and dilution VIB tube to the anaerobic quantitative agar media. The agar plates will be spread with a glass rod.

(1) The aerobic quantitative media for the Hardware and Clothing swabs include:

(a) Blood agar

(2) The anaerobic quantitative media for the Hardware and Clothing swabs include:

(a) Blood agar with vitamin K and Hemin

(3) Four milliliters from each TSB sample tube will be aseptically transferred to a labelled sterile screw-cap tube for mycobiological analysis.

c. Incubation and Quantitation:

(1) The aerobic quantitative media will be incubated at 35 C for 48 hours.

(2) The anaerobic quantitative media will be incubated at 35 C for 96 hours under an atmosphere of hydrogen gas.

(3) Colony counts will be performed on all quantitative media after incubation.

d. Isolation Streaks:

(1) After mycological samples have been removed from the TSB sample tubes, the TSB and VIB sample tubes will be incubated for 24 hours at 35 C. In addition, all TSB and VIB

dilution tubes will be [illegible] hours at 35 C and stored at 4 C for 7 days.

(2) After incubation a loop will be used to transfer culture from each sample tube to the isolation media. An isolation streak will be made on [illegible].

(3) The isolation media used for the Hardware and Clothing samples include:

(a) Blood agar

(b) MacConkey agar

(c) Blood agar with vitamin K and Hemin (Anaerobic)

(4) The streaked isolation [illegible] will be incubated for 48 or 96 hours at 35 C under the appropriate atmosphere.

(5) After the Hardware and Clothing [illegible] tubes have been employed for quantitation or isolation they will be prepared for lyophilization [illegible].

D. Isolation and Identification

1. Isolation:

a. After quantitation, isolated colonies from each aerobic plate (quantitative and isolation media) will be transferred to sterile TSB. All tubes will be [illegible] and incubated at 35 C until turbid. The [illegible] will be used for staining procedures, [illegible] and storage at 4 C.

b. After quantitation, isolated colonies from each anaerobic plate (quantitative and isolation [illegible] will be transferred

to sterile Thioglycollate Broth (T[illegible] tubes will be properly identified and incubated [illegible] turbid. The Thio. pure cultures will [illegible] procedures, inoculation of biochemical media [illegible] at 4 C.

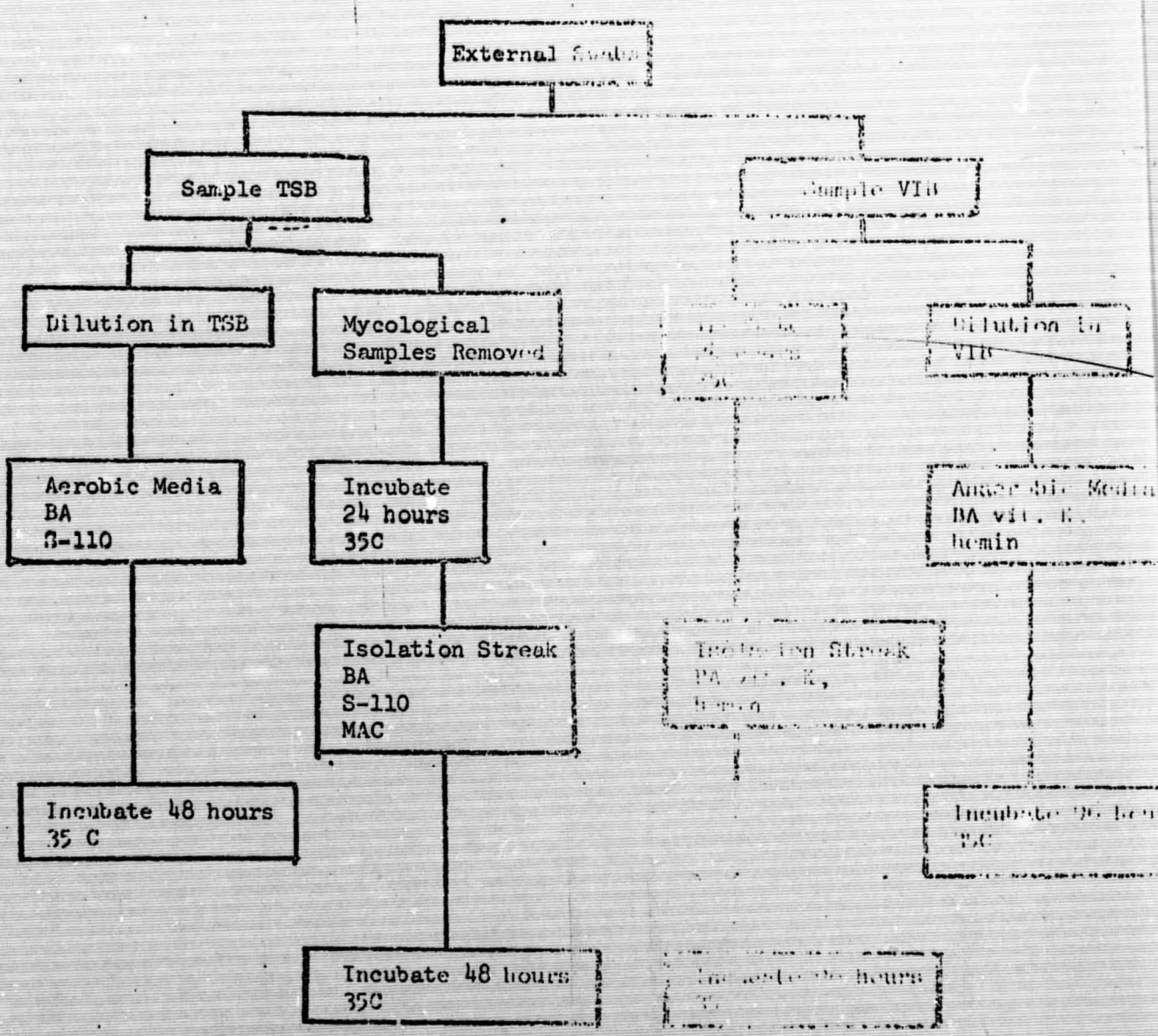
c. The isolation streak is employed [illegible] organisms which are too few to be isolated on the quantitative media. Only those organisms which were not isolated on the quantitative media will be identified. These organisms will be quantitated as <100 organisms per ml of sample. The isolation media on which the organism was cultured will be recorded.

2. Identification:

a. The pure cultures of each isolated colony (T[illegible] or Thio.) will be used to Gram stain, Spore stain, Acid-Fast stain, and to inoculate biochemical media as indicated in the following charts.

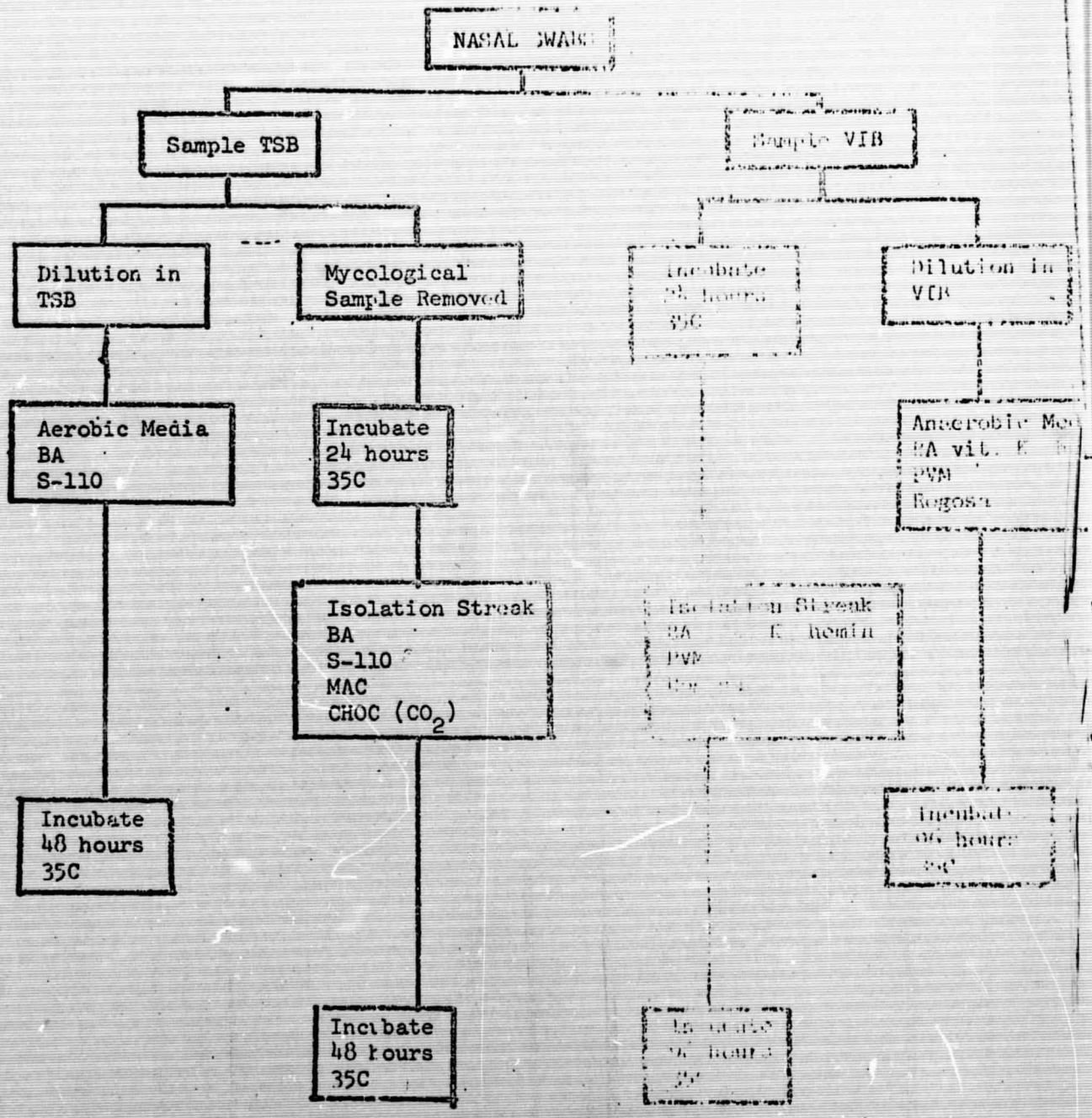
OUTLINE OF BACTERIOLOGICAL [illegible]

EXTERNAL SWABS



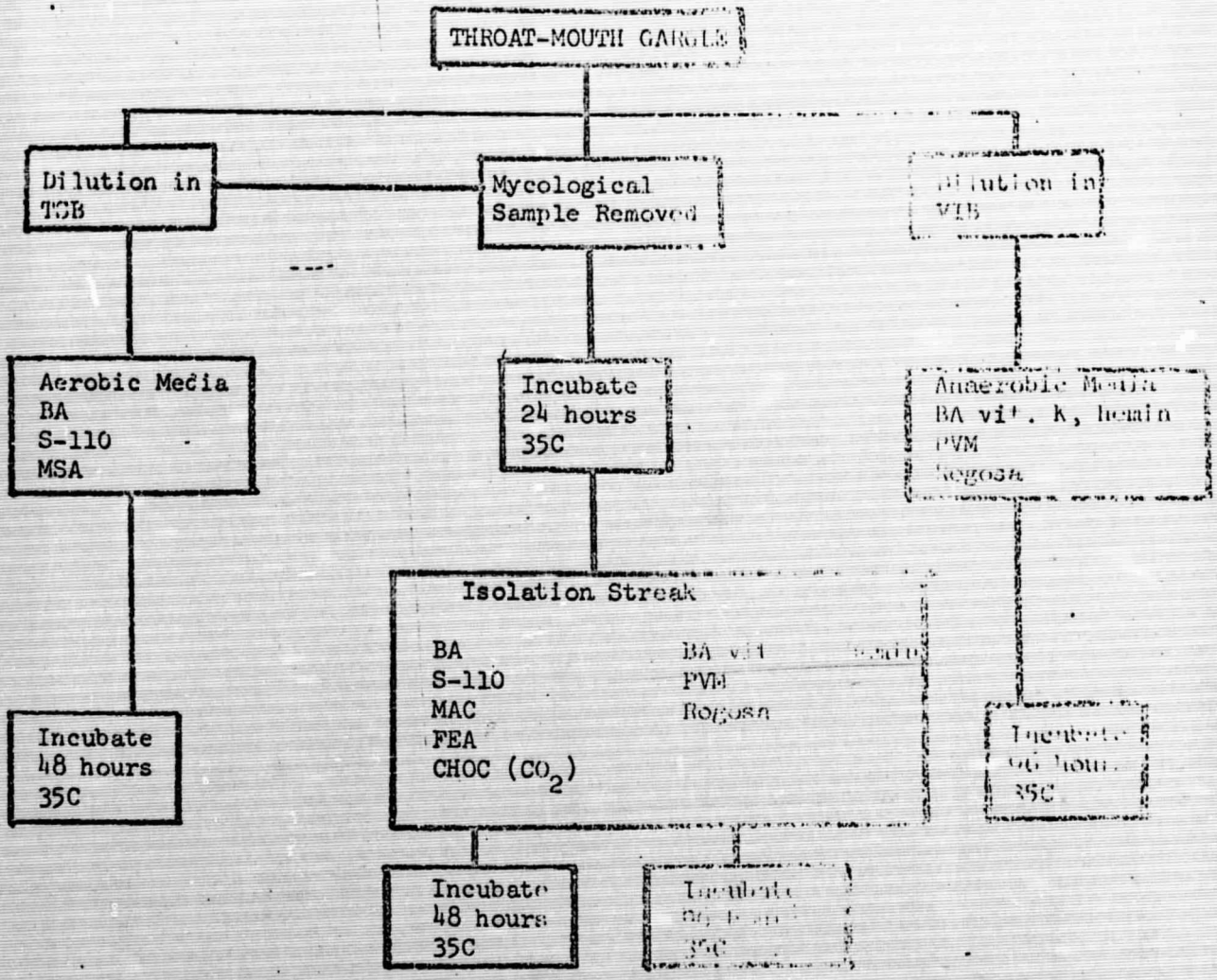
OUTLINE OF BACTERIOLOGICAL

NASAL SWABS



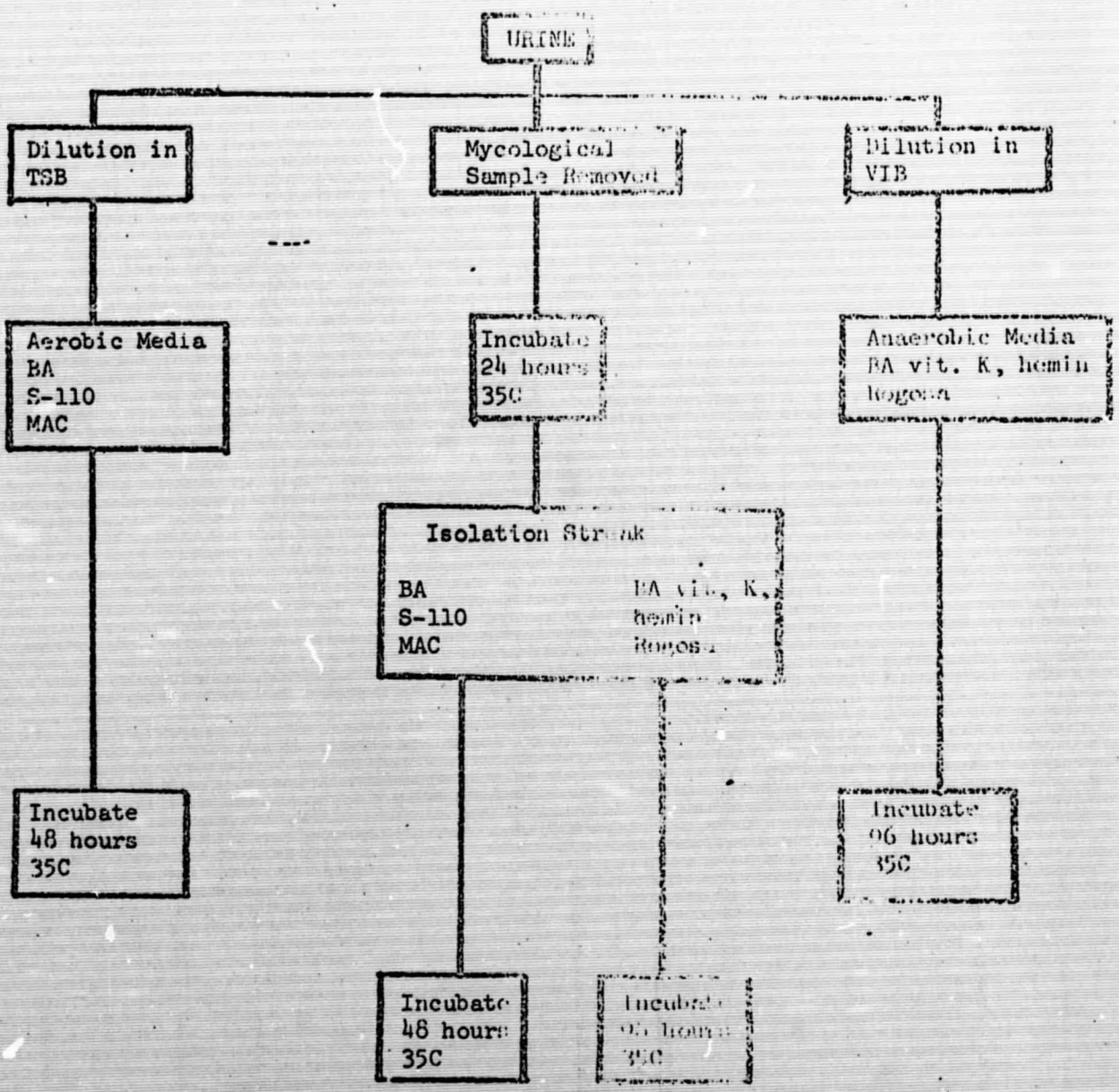
OUTLINE OF BACTERIOLOGICAL

THROAT-MOUTH GARGLE

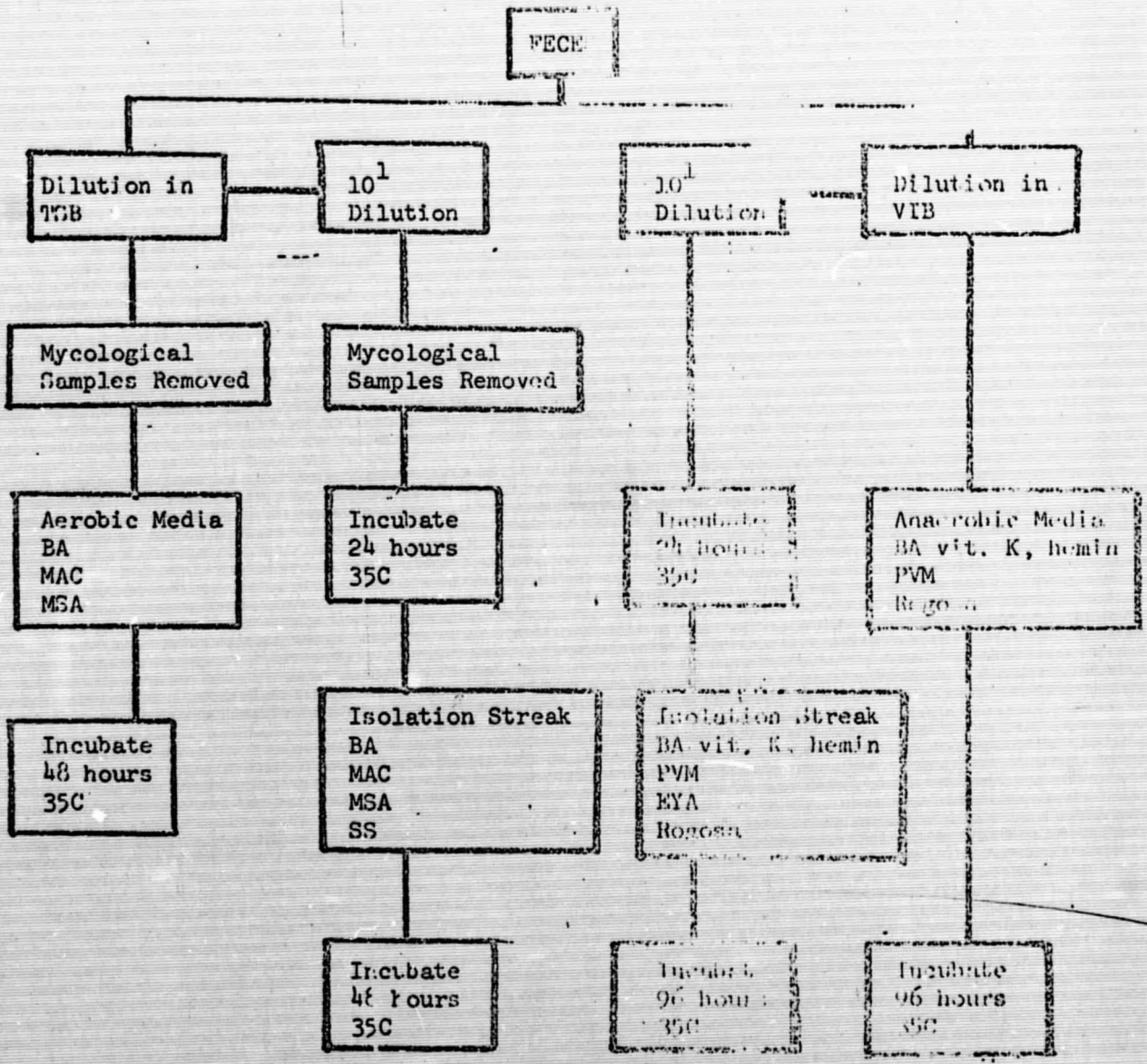


OUTLINE OF BACTERIOLOGICAL ANALYSIS

URINE

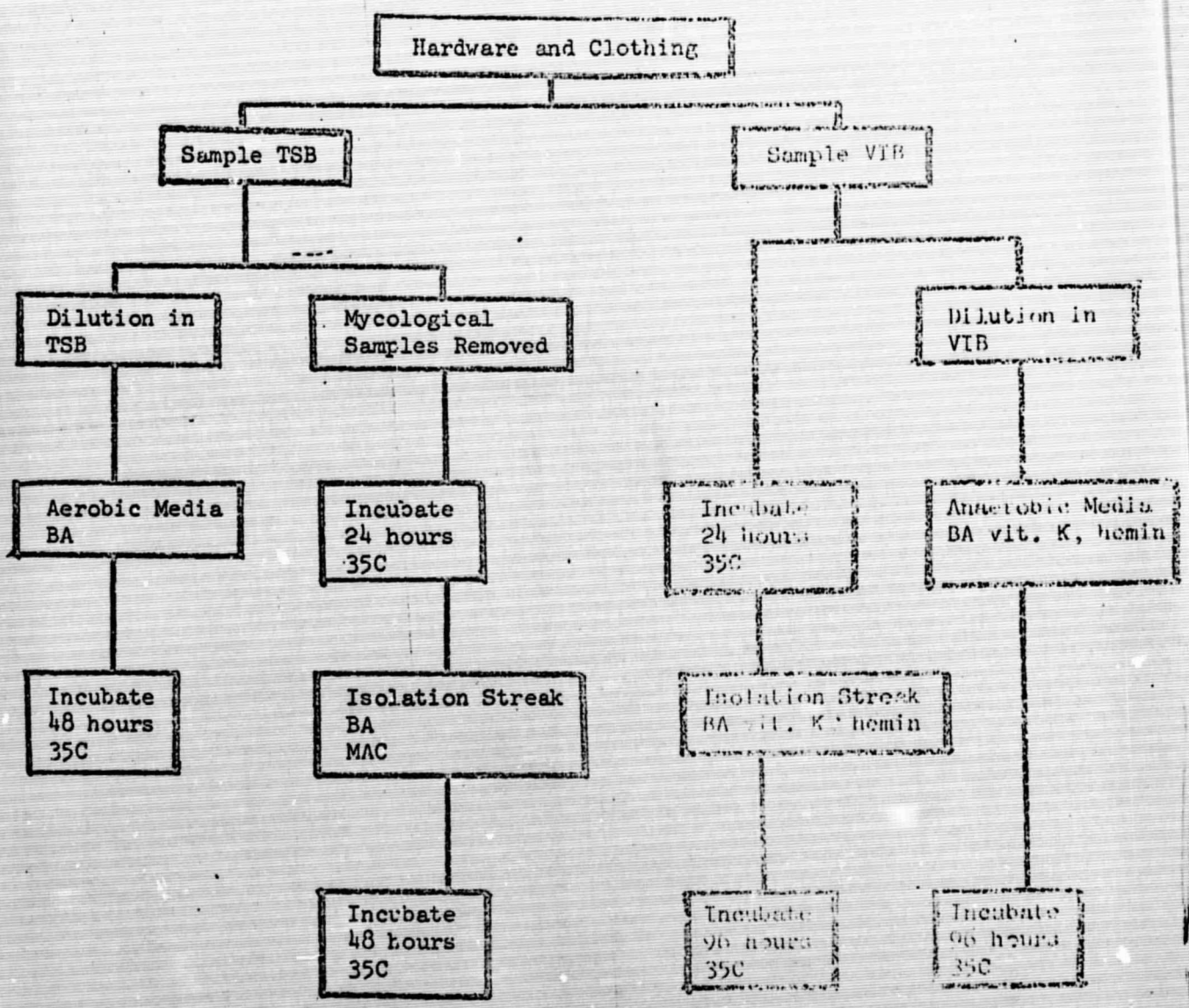


OUTLINE

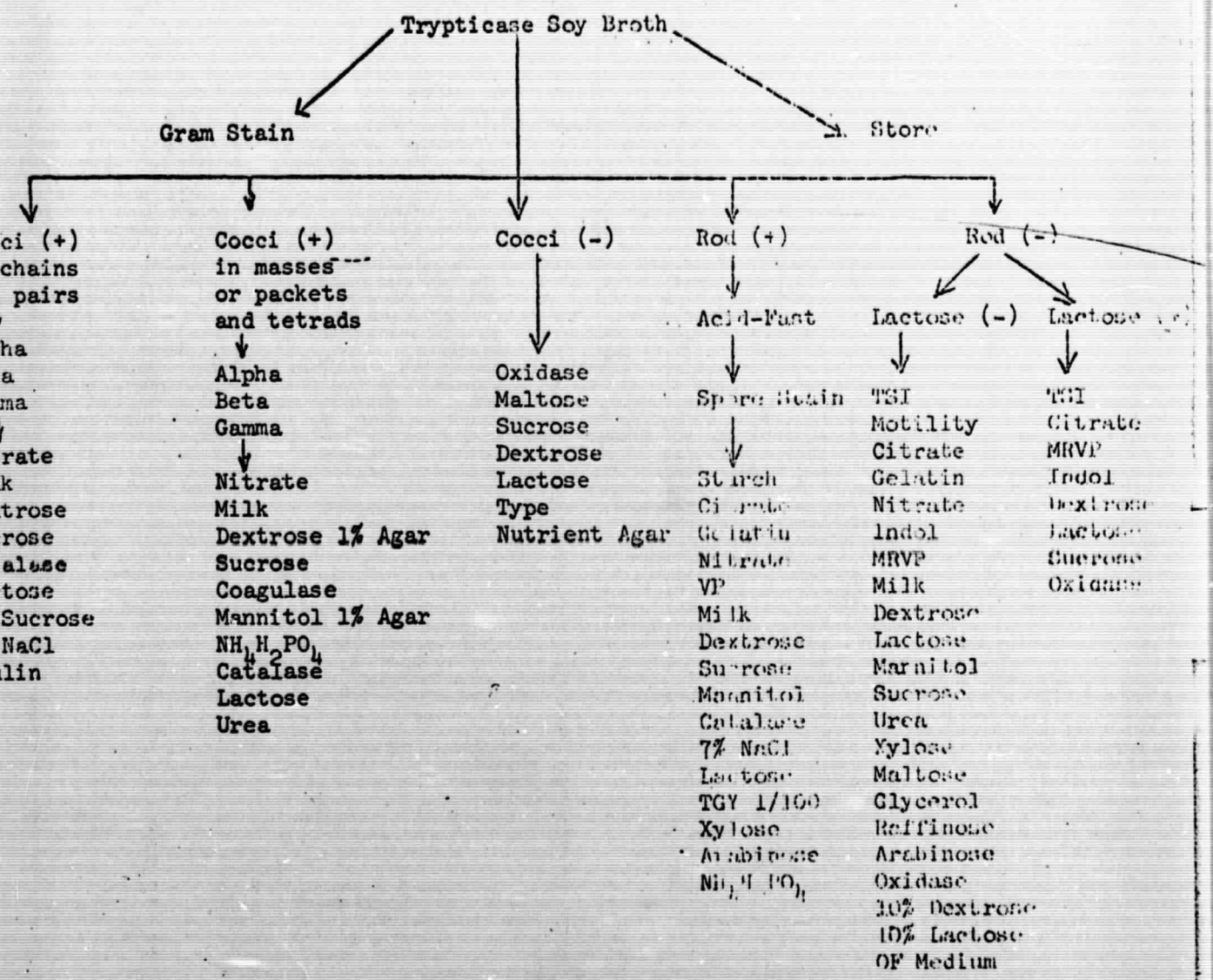

FECE
Dilution in TGB
10^1 Dilution
10^1 Dilution
Dilution in VIB
Mycological Samples Removed
Mycological Samples Removed
Aerobic Media BA MAC MSA
Incubate 24 hours 35C
Incubate 24 hours 35C
Anaerobic Media BA vit. K, hemin PVM Rogosa
Incubate 48 hours 35C
Isolation Streak BA MAC MSA SS
Isolation Streak BA vit. K, hemin PVM EYA Rogosa
Incubate 48 hours 35C
Incubate 96 hours 35C
Incubate 96 hours 35C

OUTLINE OF BACTERIOLOGICAL ANALYSIS

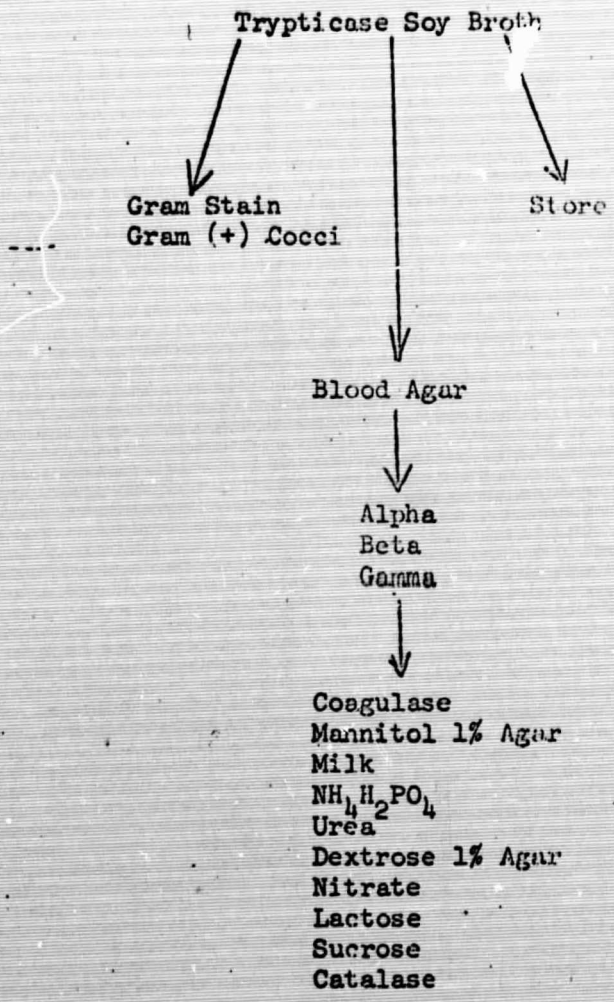
HARDWARE AND CLOTHING



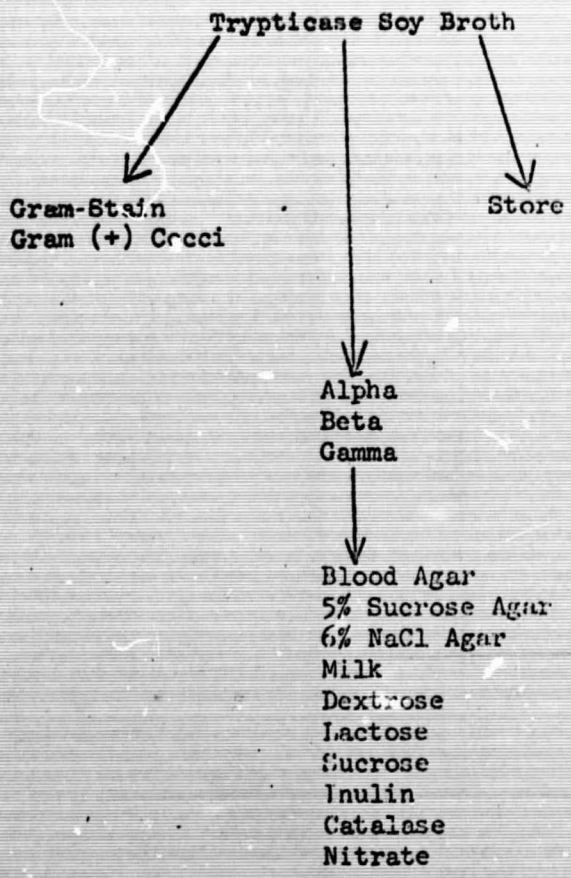
AEROBIC BLOOD AGAR SCHEME



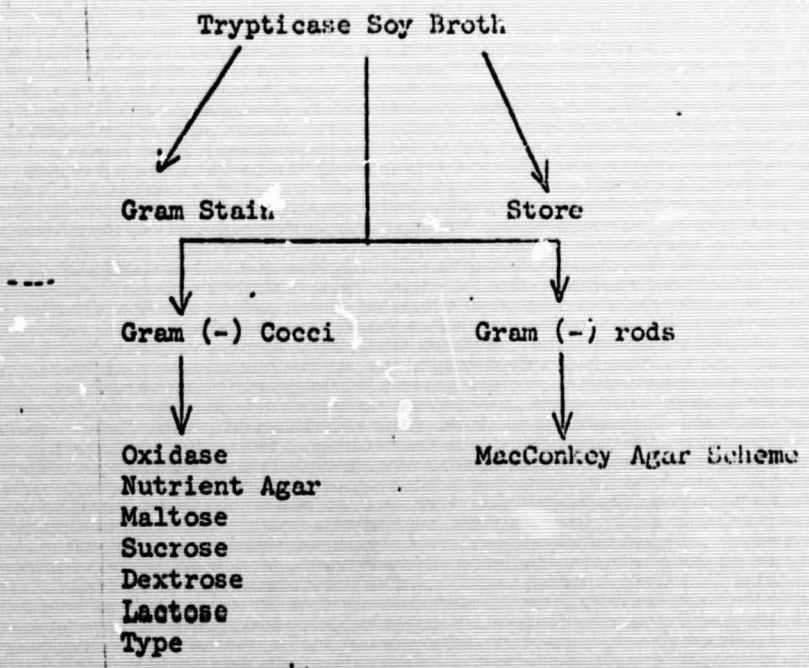
STAPHYLOCOCCUS-110 AGAR SCHEME



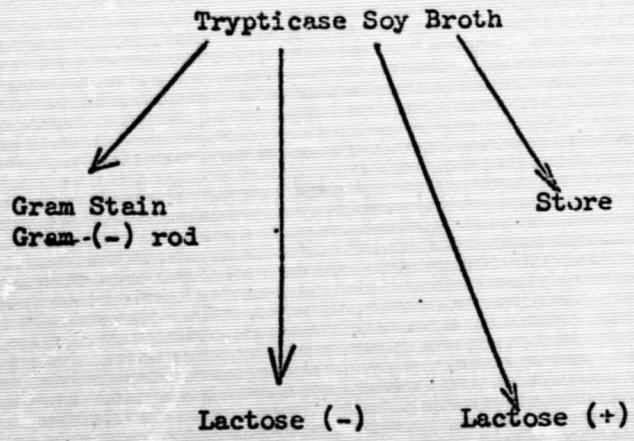
MITIS-SALIVARIUM AGAR SCHEME



CHOCOLATE AGAR SCHEME



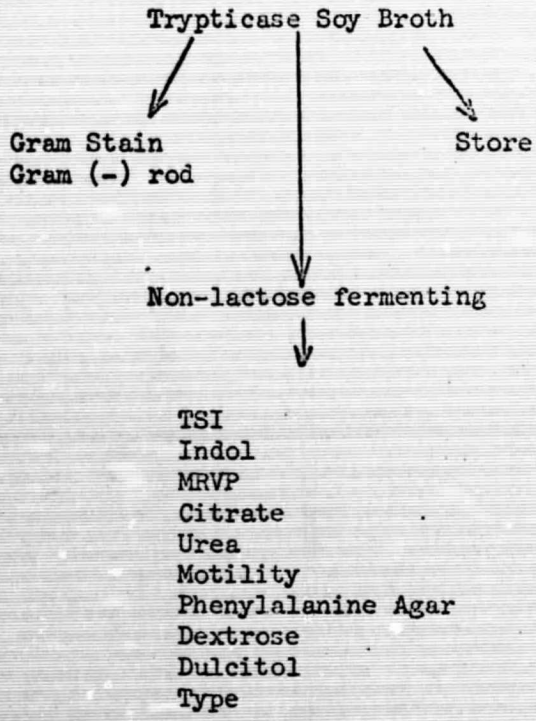
MACCONKEY AGAR SCHEME



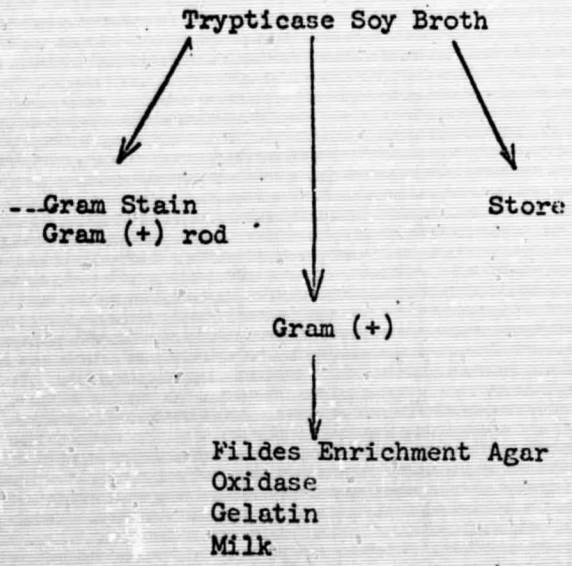
TSI
Motility
Citrate
Gelatin
Nitrate
Indol
MRVP
Milk
Dextrose
Lactose
Mannitol
Sucrose
Urea
Xylose
Maltose
Glycerol
Raffinose
Arabinose
Oxidase
10% Dextrose
10% Lactose
OF Medium

TSI
Citrate
MRVP
Indol
Dextrose
Lactose
Sucrose
Oxidase

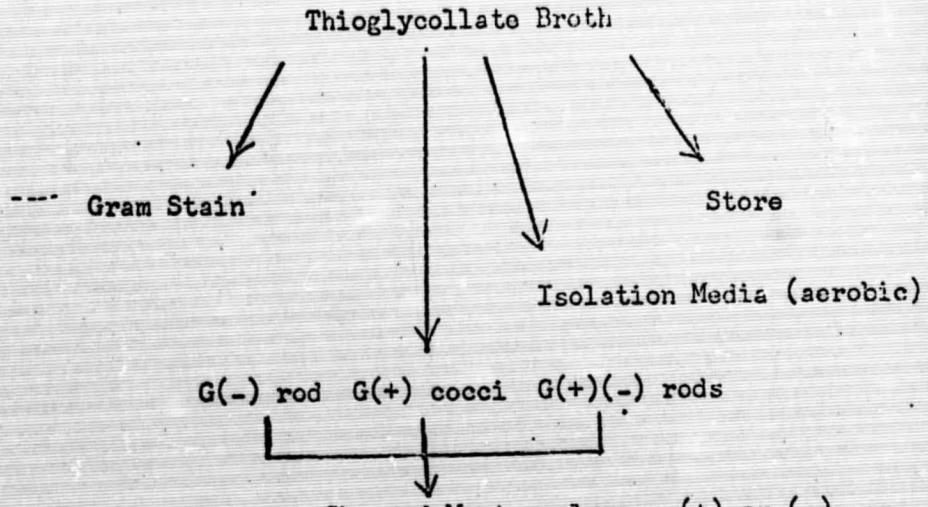
SALMONELLA-SHIGELLA AGAR SCHEME



FILDES ENRICHMENT AGAR SCHEME



ANAEROBIC SCHEME



Chopped Meat - glucose (+) or (-)
Glucose
Mannitol
Lactose
Sucrose
Maltose
Salicin
Glycerol
Starch
Xylose
Arabinose
Gelatin
Milk
Indol
NO_2
TSI
Urea
Motility
Esculin
HIA

Division II

Mycology

A. Throat-Mouth Gargle and Feces Samples:

All Throat-Mouth Gargle and Feces 4 ml samples and 4 ml TSB dilution samples will be transferred to the Mycology Area as soon as possible.

1. Throat-Mouth Gargle:

a. One-tenth milliliter aliquotes will be removed from the Throat-Mouth Gargle sample bottles and the 10^1, 10^2, and 10^3 TSB dilution tubes and transferred to each quantitative media:

(1) Corn Meal-Malt Extract agar* (CMMY)

(2) Sabourauds Dextrose agar* (SAB)

b. The plates will be spread with a glass rod and incubated at 25 C for 120 hours.

c. Four milliliters of the Throat-Mouth Gargle samples will each be aseptically transferred to a sterile centrifuge tube. The sample will be centrifuged at 5000 rpm for 15 minutes.

d. The supernate will be discarded and a swab used to sample the bottom of the centrifuge tube.

e. The swab will be used to streak each of the isolation media:

(1) CMMY

(2) SAB

f. The streaked plates will be incubated at 25 C for 120 hours.

2. Feces:

a. One-tenth milliliter aliquotes will be removed from the 10^1, 10^2, 10^3, and 10^4 TSB stool dilution tubes and transferred

* Contains antibiotics

to each quantitative media:

(1) CMMY

(2) SAB

b. The plates will be spread with a glass rod and incubated at 25 C for 120 hours.

c. Four milliliters of the 10^1 TSB stool dilution tubes will each be aseptically transferred to a sterile centrifuge tube. The samples will be centrifuged at 5000 rpm for 15 minutes.

d. The supernate will be discarded and a swab used to sample the bottom of the centrifuge tube.

e. The swab will be used to streak each of the isolation media:

(1) CMMY

(2) SAB

f. The streaked plates will be incubated at 25 C for 120 hours.

B. Crew External Swabs, Urine, Spacecraft Hardware and Clothing:

All Crew External Swabs, Urine, Spacecraft Hardware, and Clothing 4 ml sample tubes will be transferred to the Mycology Area as soon as possible.

1. Scalp, External Auditory Canal, Axilla, Umbilicus, Inguinal Region, Toe Webs, Hands, Nasal Passages, Floor, Maneuvering Knob, Drink-gun, Urine Collection Device, Gloves, and Shoe Soles:

a. Four milliliters of the Crew External, Spacecraft Hardware, and Clothing, 10^1 TSB dilution tubes will each be aseptically transferred to a sterile centrifuge tube. The sample will be centrifuged at 5000 rpm for 15 minutes.

b. The supernate will be discarded and a swab used to sample the bottom of the centrifuge tube.

c. The swab will be used to streak each of the isolation media:

(1) CMMY

(2) SAB

d. The streaked plates will be incubated at 25 C for 120 hours.

2. Urine:

a. Four milliliters of the undiluted urine samples will each be aseptically transferred to a sterile centrifuge tube. The sample will be centrifuged at 5000 rpm for 15 minutes.

b. The supernate will be discarded and a swab used to sample the bottom of the centrifuge tube.

c. The swab will be used to streak each of the isolation media.

(1) CMMY

(2) SAB

d. The streaked plates will be incubated at 25 C for 120 hours.

C. Identification:

Mycological species isolated from the Throat-Mouth Gargle and the Feces will be quantitated when feasable. All Mycological species isolated will be identified according to the outline on the following page.